Exploring protein energy landscapes

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6

OPTICAL DEPHASING IN ZN-MYOGLOBIN

6.1 Introduction

The previous chapter has dealt with protein fluctuations that occur on time scales longer than microseconds. The purpose of this section is to provide some insight into dynamic processes that take place on shorter time scales. We adopt the same approach as in chapter 5 by starting off from the same quantitative description that has proven to be successful for impurity doped glasses.

As was mentioned previously, at low temperatures, the pure dephasing in glasses is assumed to be determined by the fluctuations of a random array of two-level systems. A number of studies have been devoted to relate the temporal decay of the two-pulse photon echo (2PE), and the temperature dependence of its decay rate, to the distributions of the relevant two-level system parameters. These are the tunneling matrix element, Δ, and the energy asymmetry of the two wells, ϵ. These parameters determine the distribution of flip rates, $P(R)$. Maynard et al. [*May80*] showed that an exponential decay of the 2PE requires a dipolar coupling between chromophores and two-level systems, as well as hyperbolic distribution of flip rates between roughly 10^9 and 10^{12} s^{-1}. More recently, Silbey et al. [*Sil96*] obtained expressions for the temperature dependence of the pure dephasing rate, as well as the waiting time dependence of the line width in terms of the distributions $P(\epsilon)$ and $P(\Delta)$. Assuming the expressions

$$\begin{aligned} P(\epsilon) &\sim |\epsilon|^{\mu}, \\ P(\Delta) &\sim 1/\Delta^{(1-\nu)} \end{aligned} \tag{6.1}$$

they derived the following expression for the temperature dependence of the pure dephasing rate

$$1/T_2^* \propto T^{\mu+\nu+1} \tag{6.2}$$

Furthermore, they showed that, within their model, the 2PE-signal should vary in τ, the time separation of the two pulses as

$$I_{2PE} \propto \exp[-\tau^{1-\nu/2}] \tag{6.3}$$

At higher temperatures, an exponentially activated term is often observed to contribute to the pure dephasing rate, [*Jac83*] leading to the overall expression

$$1/T_2^* = aT^{\alpha} + b\frac{\exp(-E/kT)}{1-\exp(-E/kT)} \tag{6.4}$$

The exponential term may be due to a pseudolocal mode, such as a libration of the chromophore, or a pseudolocal mode of the matrix.

6.2 Results and discussion

A. The temporal shape of the two-pulse photon echo decay

Figure 6.1 displays 2PE-decays at three different temperatures. It is immediately apparent that not all of these decays are properly described by single exponentials. Furthermore, a subtle change of the temporal shape of the decay occurs when the temperature is increased. Before interpreting this result in terms of the relevant dephasing mechanisms, it seems useful to obtain a measure for the deviation from a pure exponential decay. As it turns out, all

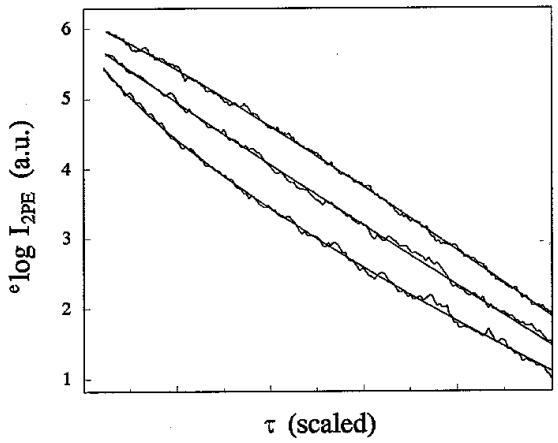

Figure 6.1 *Temporal decay of the 2PE at three different temperatures. From top to bottom: 1.9 K, 4.2 K, and 14 K. The time axes of the different decays were scaled with respect to each other to allow a comparison of their temporal shapes. The data are displayed together with fits to Eq. 6.5, yielding values of β of 1.13, 0.97, and 0.70, respectively.*

decays within the temperature range that was studied are well fitted by the decay function

$$I_{2PE}(\tau) \propto \exp[-(\tau/T_d)^{\beta}] \tag{6.5}$$

where T_d and β are constants. For $\beta < 1$ this function is usually referred to as a *stretched exponential*. Here, we only use Eq. 6.5 as a phenomenological function, where the term β characterizes the nature of the decay. We can define three borderline cases: $\beta > 1$, for which the 2PE decays slower at short τ and speeds up at longer τ, $\beta = 1$, which describes a pure exponential, and $\beta < 1$, for which the decay is faster at short τ and slows down at longer τ. Figure 6.2 shows how the fitting parameter β changes with temperature. The non-exponential character of the 2PE-decay deviates from what is generally observed for impurity doped glasses, [*Nar90*] although Meijers and Wiersma [*Mei92, 94b*] reported a slightly non-exponential 2PE-decay for Zn-porphin in deuterated ethanol glass at 1.5 K. A previous study on H_2-myoglobin, using incoherent photon echoes, [*Sai92*] showed non-exponential decays below 8 K.

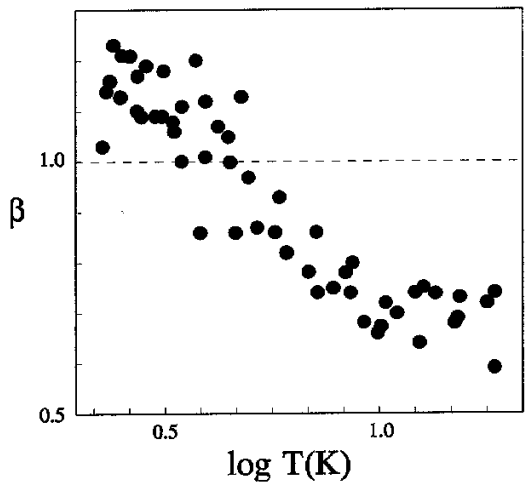

Figure 6.2 *Temperature dependence of the parameter β obtained from fits of the 2PE-decay to Eq. 6.5.*

B. The temperature dependence of the pure dephasing rate

In order to obtain some insight into the nature of the processes that contribute to the 2PE-decay, we conducted a study of the temperature dependence of the pure dephasing rate. A complication is that due to the non-exponential nature of the decays we cannot assign a unique rate constant to each decay. In order to get around this problem we force-fitted each

decay to a single exponential. The results of this experiment are displayed in Fig. 6.3. The solid line through the data is a fit to Eq. 6.4, from which we obtain $\alpha = 1.09$, $\Delta E = 40$ cm^{-1}, $a = 0.141$ GHz, and $b = 145$ GHz. Note that it is assumed that the dephasing rate should extrapolate to its lifetime limited value for $T \to 0$. T_1 was measured to be 2.0 ns, using a time correlated single photon counting set-up. [*Boe90*]

If we momentarily ignore the non-exponential relaxation of the 2PE-decay, we observe that the temperature dependence of the pure dephasing of myoglobin is very similar to that of glasses, as also can be concluded from hole burning measurements. [*Gaf95b, Box87*] The hole burning experiments yielded slightly higher values of the parameter α, between 1.3 and 1.5, but this can be due to the contribution of slow timescale spectral diffusion. The exponentially activated term that dominates the dephasing above 10 K is not unexpected, as the low frequency pseudolocal modes that are observed in molecular crystals and glasses are likely to have their counterparts in proteins. However, the fact that proteins and glasses behave in a very similar fashion at low temperature is rather surprising, in particular if we consider the results of chapter 5. A temperature dependence of the line width that can be described by a single power law over a fairly large temperature range requires that the distributions of two-level system parameters are both broad and featureless, which stands in sharp contrast to the stepwise line broadening observed on longer timescales. It should be noted that the fluctuations observed in the previous chapter are not expected to contribute to the pure dephasing because of their small preexponential factors. This result may indicate that, apart from the features described in section 5.8, the protein energy landscape has additional features that are more similar to the energy landscape of a glass, or a polymer. For instance, transitions within the rough features observed in the low-energy areas of the energy landscape (see section 5.8) may be responsible for the pure dephasing at low temperatures. However, this is only a tentative conclusion, because we cannot ignore the possibility of

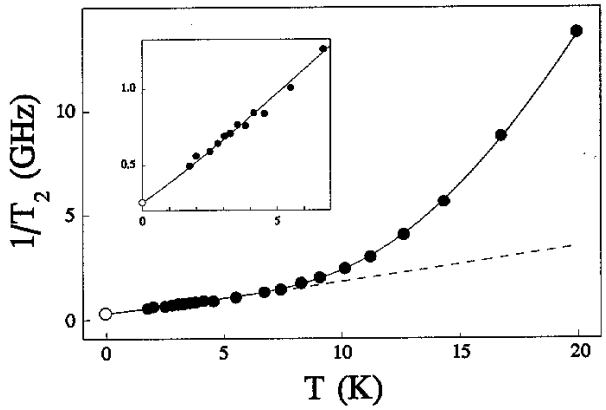

Figure 6.3 *Temperature dependence of the dephasing rate of Zn-myoglobin. The solid line is a fit to Eq. 6.4. The dashed line shows the contribution from the power law term. The insert is an enlargement of the region between 0 and 7 K. The open circle corresponds to the lifetime limited dephasing rate.*

coupling of the heme group to the two-level systems of the solvent.

Chromophores dissolved in glassy matrices exhibit spectral line broadening that is well described in terms of a dipolar coupling between the chromophores and an ensemble of spatially localized two-level systems. [*Nar90, Sil96*] The dipolar interaction strength varies as $1/r^3$, r being the distance between the chromophore and the two-level system. The protein sample can be viewed as an ensemble of chromophores dissolved into a glass, where each chromophore is excluded from a small volume of the glass that is occupied by the protein. This means that the spectral diffusion experienced by the chromophore can be divided into a contribution from the protein and a contribution from the bulk solvent. Since myoglobin is a protein of small dimensions, we expect its line broadening behavior to contain a major contribution from the fluctuations of the two-level systems of the glycerol/water solvent. Therefore, if we want to investigate the dynamic behavior of the protein we need to be able to separate the contribution of the protein to the line broadening behavior from that of the solvent. For the experiments presented in chapter 5 this turned out to be a relatively straightforward task, but for the short timescale dynamics, including the line broadening observed between 10 ns and 1 µs, the situation is not quite that clear.

From chapter 5 we learned that on timescales between 1 µs and 100 ms, the solvent does not contribute to the line broadening. This observation can be interpreted in two different ways. Either the solvent does not exhibit any dynamics on these timescales, or the heme group is in some way shielded from the solvent. At first, it may seem that this problem is easily addressed by performing the same experiment on the heme molecule directly dissolved into the glycerol/water solvent. A practical problem is that Zn-mesoporphyrin IX is not soluble into glycerol/water mixtures in large enough concentrations to perform a 3PSE-experiment, and that line broadening data on different chromophores are not available. However, hole burning and photon echo studies on dye molecules, including Zn-mesoporphyrin IX (*see chapter 5*), doped into different organic glasses and polymers have shown that for practically all systems the line width continuously increases with waiting time in the region between roughly 10^{-9} and 10^{5} s. [*Koe96*] From this we may tentatively conclude that the heme group of the protein is shielded from the long timescale dynamics of the solvent. However, this does not necessarily imply that this is also the case for the short timescale dynamics. Shielding effects may be sensitive to the nature of the fluctuations which again may be correlated to the timescale on which they occur. Gafert et al. [*Gaf95b*] argued that the chromophore in the protein is completely decoupled from the solvent on the basis of Stark effect experiments. [*Gaf95c, Gaf95d*] It should be emphasized that, although these experiments clearly show that the main contribution of the matrix fields at the chromophore site arise from the protein, rather than from the glass, they do not rule out coupling to fast solvent fluctuations, which both contribute to the line width in photon echo and hole burning experiments. Summarizing, it is well possible that the low-temperature pure dephasing contains a contribution from the solvent matrix, and explanations of these results in terms of the energy landscape of the protein should be treated with caution.

C. The origin of the non-exponential two-pulse echo decay

An analysis of the non-exponential decay of the 2PE is complicated by the fact that, within a large part of the temperature range studied, there are two competing processes contributing to the pure dephasing. These are the two-level system fluctuations, and the activated mode. A possible explanation of the change of the temporal decay with temperature is a change of the relative amplitude of the two contributions, which individually can give rise to a temperature-independent decay. However, if we carefully observe Figs. 6.2 and 6.3 we must conclude that this is not the case. Figure 6.3 shows that, below 7 K, the 2PE-decay is dominated by the two-level system contribution. If we then look at Fig. 6.2, we observe that between 1.7 and 7 K already a significant change in the temporal shape of the 2PE-decay has occurred. From this we have to conclude that the two-level system contribution, by itself, gives rise to a temperature-dependent decay. Whether this is also true for the contribution of the pseudolocal mode is difficult to ascertain because it only becomes dominant at the far edge of the temperature range.

The non-exponential relaxation is not easily explained in terms of the currently available theoretical framework treating optical dephasing in disordered solids. A factor that may play a role is the two-domain nature of the sample, i.e. each chromophore is in principle both coupled to the protein and the surrounding glass. Pack et al. [*Pac90*] showed that a distinction between the solvation shell and the bulk solvent in chromophore doped glasses can lead to a non-Lorentzian line shape, or a non-exponential photon echo decay, if the density of two-level systems is different in these two regions. For a decay that can be fitted with a value of $\beta > 1$ this implies that the density of two-level systems is larger in the bulk solvent than in the solvent shell region. Pack et al. demonstrated that for a solvent shell with a radius of 20 Å, which is comparable to the dimensions of myoglobin, a strongly non-exponential echo decay ($\beta > 1$) would result if the density of two-level systems inside the solvent shell is zero. As the measured 2PE decays are only slightly non-exponential, this leads us to conclude that the protein at least to some degree contributes to the pure dephasing. The two-domain nature of the sample may also play a role in the temperature dependence of the temporal decay of the 2PE. This behavior may be explained by a different temperature dependence of the concentration of thermally active two-level systems for the glass and the protein. The fact that $\beta > 1$ below 5 K and $\beta < 1$ above 5 K would imply that below 5 K the density of thermally active two-level systems is larger in the glass than in the protein, and that around 5 K a crossover occurs.

It should be noted that the model of Silbey et al. is not likely to account for the non-exponential echo relaxation. In terms of this model the non-exponential relaxation of the 2PE implies that the parameter ν is not equal to zero, which is well possible. However, its value is not expected to change with temperature. An alternative explanation for the change of the 2PE-decay with temperature is in terms of a distribution of fluctuation rates that does not uniformly decay towards higher rates, such as a hyperbolic function, but peaks at a certain

value, such as the log-normal distribution functions that were introduced by Jankowiak et al. [*Jan86*] The 2PE-decay is sensitive to fluctuations occurring in the region between roughly 10^9 and 10^{12} s^{-1}. A value of β larger than 1 implies that the amplitude of the log-normal function in the relevant region is smaller at high rates, consistent with a distribution that peaks at the slow end of the relevant region of fluctuation rates. [*Mei94b*] If the temperature is increased, the maximum of the distribution will shift, so that eventually it peaks at the fast end of the relevant region. In this case, the amplitude of the distribution is larger for high fluctuation rates than for low ones, resulting in a stretched exponential decay, i.e. $\beta < 1$. A problem is that a log-normal distribution does not agree with the nearly linear dependence of the dephasing rate on temperature, which points towards a hyperbolic distribution of fluctuation rates, i.e. $P(R) = \omega/R$.

The discussion of the shape of the distribution of relaxation rates also bears relevance to the short timescale spectral diffusion observed in chapter 5. For glasses it is often assumed that a uniform distribution of relaxation rates accounts for both the 2PE-decay and the waiting time dependence of line width. [*Sil96*] Based on the fact that the observed 2PE-decay of Zn-myoglobin cannot be explained in terms of the discrete features that describe the long timescale line broadening, we need to conclude that this assumption does not hold for the protein. However, it seems plausible that the short timescale line broadening arises from the same distribution that determines the 2PE-decay. This would imply that the short timescale spectral diffusion is either due to more random, glass-like features of the protein energy landscape, or to coupling of the chromophore with the solvent.

Maynard et al. [*May80*] showed that for a hyperbolic distribution the pure dephasing rate can be expressed in terms of the coupling constant, ω, as

$$1/T_2^* = 3.66\,\omega \tag{6.6}$$

If we compare the values of ω obtained from fits of the short time scale spectral diffusion to a cut off hyperbolic function (*see section 5.4*) to the pure dephasing rate obtained from the 2PE-measurements we observe that the decay rate of the 2PE that is predicted by Eq. 6.6 is always larger than the one that is experimentally observed. Assuming that the pure dephasing and the short time scale spectral diffusion arise from the same class of dynamics this may be considered as further evidence in favor of a log-normal distribution.

Finally, the non-exponential relaxation due to the pseudolocal mode my be easily explained in terms of a distribution of activation energies, rather than a single activation energy, which would yield a stretched exponential decay. Although the exact nature of this mode remains uncertain, the fact that a distribution of energies exists may indicate a strongly local character, such as a librational mode of the heme group within its pocket. A distribution of activation energies would be consistent with a distribution of local structures, which is in agreement with the large degree of structural indeterminism observed in proteins.

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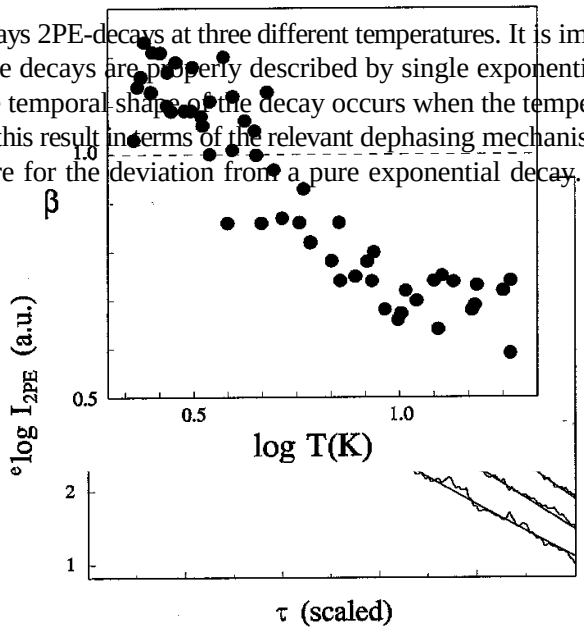

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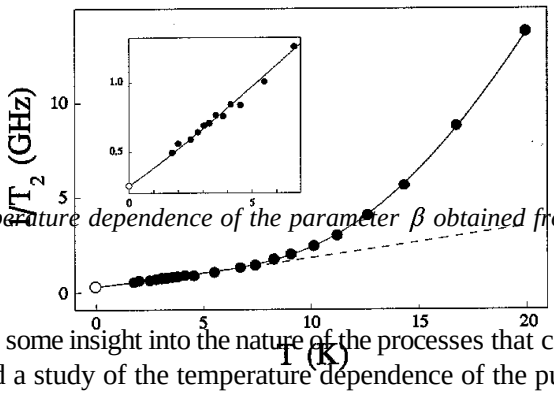

Figure 6.2 *Temperature dependence of the parameter β obtained from fits of the 2PE-decay to Eq. 6.5.*

In order to obtain some insight into the nature of the processes that contribute to the 2PE-decay, we conducted a study of the temperature dependence of the pure dephasing rate. A complication is that due to the non-exponential nature of the decays we cannot assign a unique rate constant to each decay. In order to get around this problem we force-fitted each decay to a single exponential. The results of this experiment are displayed in Fig. 6.3. The

solid line through the data is a fit to Eq. 6.4, from which we obtain $\alpha = 1.09$, $\Delta E = 40$ cm^{-1}, $a = 0.141$ GHz, and $b = 145$ GHz. Note that it is assumed that the dephasing rate should extrapolate to its lifetime limited value for $T \to 0$. T_1 was measured to be 2.0 ns, using a time correlated single photon counting set-up. [*Boe90*]

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Figure 6.3 *Temperature dependence of the dephasing rate of Zn-myoglobin. The solid line is a fit to Eq. 6.4. The dashed line shows the contribution from the power law term. The insert is an enlargement of the region between 0 and 7 K. The open circle corresponds to the lifetime limited dephasing rate.*

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C. The origin of the non-exponential two-pulse echo decay

An analysis of the non-exponential decay of the 2PE is complicated by the fact that, within a large part of the temperature range studied, there are two competing processes contributing to the pure dephasing. These are the two-level system fluctuations, and the activated mode. A possible explanation for the change of the temporal decay with temperature is a change of the relative amplitude of the two contributions, which individually can give rise to a temperature-independent decay. However, if we carefully observe Figs. 6.2 and 6.3 we must conclude that this is not the case. Figure 6.3 shows that, below 7 K, the 2PE-decay is dominated by the two-level system contribution. If we then look at Fig. 6.2, we observe that between 1.7 and 7 K already a significant change in the temporal shape of the 2PE-decay has occurred. From this we have to conclude that the two-level system contribution, by itself, gives rise to a temperature-dependent decay. Whether this is also true for the contribution of the pseudolocal mode is difficult to ascertain because it only becomes dominant at the far edge of the temperature range.

The non-exponential relaxation is not easily explained in terms of the currently available theoretical framework treating optical dephasing in disordered solids. A factor that may play a role is the two-domain nature of the sample, i.e. each chromophore is in principle both coupled to the protein and the surrounding glass. Pack et al. [*Pac90*] showed that a distinction between the solvation shell and the bulk solvent in chromophore doped glasses can lead to a non-Lorentzian line shape, or a non-exponential photon echo decay, if the density of two-level systems is different in these two regions. For a decay that can be fitted with a value of $\beta > 1$ this implies that the density of two-level systems is larger in the bulk solvent than in the solvent shell region. Pack et al. demonstrated that for a solvent shell with a radius of 20 Å, which is comparable to the dimensions of myoglobin, a strongly non-exponential echo decay ($\beta > 1$) would result if the density of two-level systems inside the solvent shell is zero. As the measured 2PE decays are only slightly non-exponential, this leads us to conclude that the protein at least to some degree contributes to the pure dephasing. The two-domain nature of the sample may also play a role in the temperature dependence of the temporal decay of the 2PE. This behavior may be explained by a different temperature dependence of the concentration of thermally active two-level systems for the glass and the protein. The fact that $\beta > 1$ below 5 K and $\beta < 1$ above 5 K would imply that below 5 K the density of thermally active two-level systems is larger in the glass than in the protein, and that around 5 K a crossover occurs.

It should be noted that the model of Silbey et al. is not likely to account for the non-exponential echo relaxation. In terms of this model the non-exponential relaxation of the 2PE implies that the parameter ν is not equal to zero. This is well possible, but its value is not expected to change with temperature. An alternative explanation for the change of the 2PE-decay with temperature is in terms of a distribution of fluctuation rates that does not uniformly decay towards higher rates, such as a hyperbolic function, but peaks at a certain

value, such as the log-normal distribution functions that were introduced by Jankowiak et al. [*Jan86*] The 2PE-decay is sensitive to fluctuations occurring in the region between roughly 10^9 and 10^{12} s^{-1}. A value of β larger than 1 implies that the amplitude of the log-normal function in the relevant region is smaller at high rates, consistent with a distribution that peaks at the slow end of the relevant region of fluctuation rates. [*Mei94b*] If the temperature is increased, the maximum of the distribution will shift, so that eventually it peaks at the fast end of the relevant region. In this case, the amplitude of the distribution is larger for high fluctuation rates than for low ones, resulting in a stretched exponential decay, i.e. $\beta < 1$. A problem is that a log-normal distribution does not agree with the nearly linear dependence of the dephasing rate on temperature, which points towards a hyperbolic distribution of fluctuation rates, i.e. $P(R) = \omega/R$.

The discussion of the shape of the distribution of relaxation rates also bears relevance to the short time scale spectral diffusion observed in chapter 5. For glasses it is often assumed that a uniform distribution of relaxation rates accounts for both the 2PE-decay and the waiting time dependence of line width. [*Sil96*] Based on the fact that the observed 2PE-decay of Zn-myoglobin cannot be explained in terms of the discrete features that describe the long time scale line broadening, we need to conclude that this assumption does not hold for the protein. However, it seems plausible that the short time scale line broadening arises from the same distribution that determines the 2PE-decay. This would imply that the short time scale spectral diffusion is either due to more random, glass-like features of the protein energy landscape, or to coupling of the chromophore with the solvent.

Maynard et al. [*May80*] showed that for a hyperbolic distribution the pure dephasing rate can be expressed in terms of the coupling constant, ω, as

$$1/T_2^* = 3.66\,\omega \tag{6.6}$$

If we compare the values of ω obtained from fits of the short time scale spectral diffusion to a cut off hyperbolic function (*see section 5.4*) to the pure dephasing rate obtained from the 2PE-measurements we observe that the decay rate of the 2PE that is predicted by Eq. 6.6 is always smaller than the one that is experimentally observed. Assuming that the pure dephasing and the short time scale spectral diffusion arise from the same class of dynamics this may be considered as further evidence in favor of a log-normal distribution.

Finally, the non-exponential relaxation due to the pseudolocal mode my be explained in terms of a distribution of activation energies, rather than a single activation energy. This would result in a stretched exponential decay. Although the exact nature of this mode remains uncertain, the fact that a distribution of energies exists may indicate a strongly local character, such as a librational mode of the heme group within its pocket. A distribution of activation energies would be consistent with a distribution of local structures, which is in agreement with the large degree of structural indeterminism observed in proteins.